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Histopathological changes in the gonads of Male rabbits (*Oryctolagus cuniculus*) on exposure to imidacloprid insecticide

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Abstract

The present study was aimed to know the reproductive effects of imidacloprid, a neonicotinoid insecticide on the gonads of male rabbits. In this context, a total of 30 male rabbits, *Oryctolagus cuniculus*, were randomly divided into three groups (ten rabbits per group) as two test groups and one group labeled as a control. $1/10^{\text{th}}$ of LD_{50} imidacloprid was orally administered in test group animals for 10 and 20 consecutive days. After treatment a highly significant decrease ($P < 0.01$) in the body weight and ($P < 0.001$) testicular weight was recorded in both test group animals as compared to control. Thereafter, removed testes were fixed for histological examination. Histopathological observations revealed that, the interstitial space as compared to control became widened and number of leydig's cells decreased. While in 20 days treated animals such toxic effects were more pronounced. These results revealed that exposure of imidacloprid induced testicular damage in rabbits which probably can cause reproductive disorders in animals and these estimating eco toxicological hazards.

Keywords: Imidacloprid, Rabbits, Toxicity, Leydig's cell and Testicular damage

1. Introduction

Since past few decades animal population and their inhabitants have been declining throughout the world. Pesticide exposure is the major contributing factor and revealed enormous toxic effects in animals including disruption in the gonads, disorders of growth and development, immune and physiological functions. Globally varieties of agrochemicals are used in the agriculture to kill the harmful pests to increase the production. The inappropriate and indiscriminate usage of these agrochemicals is causing severe health and environmental problems. This not only harms the crops and economical pest, but the other non- target organisms and their population [1]. Currently organochloride, organophosphate, pyrethroid and neonicotinoid agrochemical compounds are used in the agriculture sector and many of them having toxic effects on reproductive organs. They cause disruption in normal system and gonads. These agrochemicals affect endocrine system and cause disruption in tissues, organs and their structures [2]. Many agrochemicals cause disturbances in the mechanism of steroidogenesis at testicular tissues [3]. Among the agrochemicals which are applied on the crops imidacloprid (IM), a neonicotinoid insecticide is commonly used in pest control [4] and it is one of the highest selling insecticides throughout the world due to its selective toxicity on pest control [5]. IM is also used for controlling fleas in various animals and birds [6]. The reported material indicates that chronic exposure to imidacloprid exhibit reproductive disorders including effects on testicular tissues and spermatogenesis which can ultimately cause infertility problems [7-10]. Studies regarding toxicity and effects of IM on reproductive organs have been documented [11, 12].

Testicular tissues are the vital organs for the production of androgen and disrupt by exposure to various agrochemicals [13]. The effects of pesticides on testicular cells have also been recognized *in vivo* [14]. Among the agrochemicals which are applied on the crops, Neonicotinoid, IM insecticide is commonly used in pest control. Therefore, health hazards of this insecticide have gained much attention worldwide due to environmental problems but narrowly studied in Pakistan.

Histopathological disruptions in animals can be used as significant biomarkers in eco-toxicology. The present study was aimed to evaluate the toxic effects of imidacloprid on testicular tissues in rabbits with a view of prospective findings to humans.

2. Materials and Methods

2.1 Animals and experimental Design

This experimental study was approved by the Advanced Studies and Research Board (ASRB) and conducted under the guidelines at Department of Zoology, University of Sindh, Jamshoro, Pakistan during September 2013- December 2013.

For evaluation of the toxic effects on testicular tissues, 30 male Rabbits *Oryctolagus cuniculus*, were housed in wooden cages (12' x 16 sq. ft.) in Endocrinology laboratory, Department of Zoology, University of Sindh, Jamshoro, Pakistan. The animals were healthy and acclimatized for 15 days prior to the start of the study. Animals were fed on grass (*Medicago sativa*) which is commonly known as Lucerne along with a free excess of water. After acclimation the rabbits were housed in the experimental cages and randomly divided in to three equal groups as two test groups and one control group. Before experimental trails body weight of control and test group animals were recorded.

2.2 Test chemical and applications

Imidacloprid 200 SL insecticide was commercially purchased from authorized dealers of Agrochemical Company. LD₅₀ oral dose was determined according to the toxicology method [15]. Test solution of insecticide was prepared at a dose of one tenth of LD₅₀ (45 mg./Kg. Body weight) in 10 ml distilled water. During experiment oral doses were administrated by disposable syringe for 10 and 20 consecutive days in each test group animal. Control group animals received distilled water for the same time period. Animals were given the same quantity of food and water.

2.3 Clinical Observations

After administration of doses detailed observations were made on a daily basis for 4 to 5 hours. During observations the parameters of adverse effects, diet intake, behavioral changes and mortality were assessed. At the termination of the experiment on day ten and fifteen, body weight of treated and control rabbits were recorded. Thereafter, all animals were killed through anesthetized procedure and dissected to remove the gonads (testes). The testicular weight was noted in the treated and control group rabbits.

2.4 Histological procedure

Pieces of removed testes were fixed for 24 hours in Bouin's fixative for histopathological studies. Fixed material was washed out for 3-5 minutes in running tap water for removal of excessive fixative solution. After that pieces of testes were passed through alcohol series for dehydration procedure and tissues were embedded in paraffin wax followed by 6µm thick sections were cut by using rotary microtome machine. Harris's hematoxylin (Gurr, 1956) & Eosin (Putt, 1948) staining procedure was used in the staining of slides.

2.5 Statistical analysis

Data was statistically analyzed by one way ANOVA test on (IBM SPSS, 19) program followed by Least Significance Test (LSD) for comparisons between groups. Values articulated as Mean ± SD and P values < 0.05 were considered statistically significant.

3. Results and Discussion

The reduction in the testes, body weight and hypertrophy in leydig's cells in the animals exposed to imidacloprid (IM) has been observed along with decreased movement, trembling, diarrhea congested interstitial space, atrophied seminiferous tubules and decreased number of leydig's cells was also noted by the exposure of imidacloprid [16]. IM treatment has been reported to cause decline in the body weight of exposed animals [17]. Adverse effect on body growth is the most common clinical symptom of IM in treated animals [18]. In the present study similar results regarding body weight, testicular weight and clinical symptoms in the treated animals were recorded. Results clearly indicated that exposure to IM exhibited highly significant decrease ($P < 0.01$) in body weight and ($P < 0.001$) in testicular weight respectively in both test groups as compared to control (Table 01). Absorption of IM takes place in the gastrointestinal tract [19] and affects multiple organs [20]. Behavioral changes and other health problems like decreased movement, trembling and diarrhea in animals were observed when exposed to IM [21]. Toxicity of IM in animals is seen as fatigue, diarrhea, weakness, cramps, tremor, and convulsion [22]. Our results revealed that, visible signs of IM toxicity exerted less adverse effects on food intake in treated animals during experimental period. Hence animals were relatively inactive and showed clear signs of toxicity in the treated animals such as fatigue, trembling, convulsion, dizziness, Tremors, diarrhea and behavioral changes in relation to control group (Fig. 01). Testosterone is produced by leydig's cells of the testes and it is the most important male hormone for the development of sexual characteristics [23]. Hypogonadism resulting with defective seminiferous tubules and leydig's cells function may cause infertility in animals due to reduced production of spermatogenic cells [24]. There are numerous studies suggesting that, chronic exposure of IM exhibit defects in the gonads of animals [25]. In the present study histopathological observations confirmed that, IM caused testicular damage in test group animals. Animals treated for 10 days showed empty seminiferous tubules in the lumen and interstitial space became widened, probably due to elimination of leydig's cells (Fig. 4) and in few tubules this space was compressed. Picnosis in the spermatocytes and vacuolation in the seminiferous tubules was observed (Fig. 5). Whereas histological structure of testis in the control animals demonstrated that, seminiferous tubules were proper sized and oval/rounded shaped with appearance of appropriate and narrow interstitial space. Seminiferous tubules containing different types of spermatogenic cells and sertoli cells were observed (Figs. 2-3). IM and many other neonicotinoid agrochemicals could cause potential effects on reproductive organs in animals like disruption of testicular tissues and spermatogenic cells and decline in sperm motility [26]. Low dose exposure of neonicotinoid insecticide had moderate effects on the gonads and could be more severe at higher doses so that the reproductive system can be more vulnerable to exposure of neonicotinoids [27]. In agreement with earlier findings our histopathological findings revealed that, 20 days exposure of IM exerted conspicuous changes in the testicular structure with severe congestions in interstitial space. Only few normal sized leydig's cells were present suggesting that they were under stress and could be destroyed at any time (Fig. 6). Insecticides like IM are the reproductive toxicants in male animals and these may induce necrosis in the seminiferous tubules along with histopathological alterations [28-30]. Few of the seminiferous tubules became elongated due to hypertrophy and no leydig's cells were present in the interstitial space (Fig. 07). Results of present study suggest that exposure to

imidacloprid can be a potential cause of histological damage in the testes of animals which may lead to infertility problems and undermine the ability to reproduce. Our results also revealed the hazardous effects of IM exposure on mammalian fertility as well

raising the concern that imidacloprid may cause reproductive risks on human reproductive organs.

Table 1: Showing Parameters Examined in the Control and treated Rabbits

Animals (Rabbits)	No. of Rabbits n = 30	Body weight (Mean±S.D) Grams	Testes weight (Mean±S.D) Grams
Control	10	1286±39.43 ^a	4.10±0.06 ^a
Treated (10 Days)	10	1181±29.29 ^{**b}	3.62±0.10 ^{***b}
Treated (20 days)	10	1085±32.32 ^{**c}	3.24±0.08 ^{***c}

The table indicates the value with (Mean ± SD) body & testes weight in treated rabbits with highly significant (P < 0.01) ** and (P < 0.001) *** decrease respectively as compared to control group. Mean values not sharing same letters are significantly different with each other.

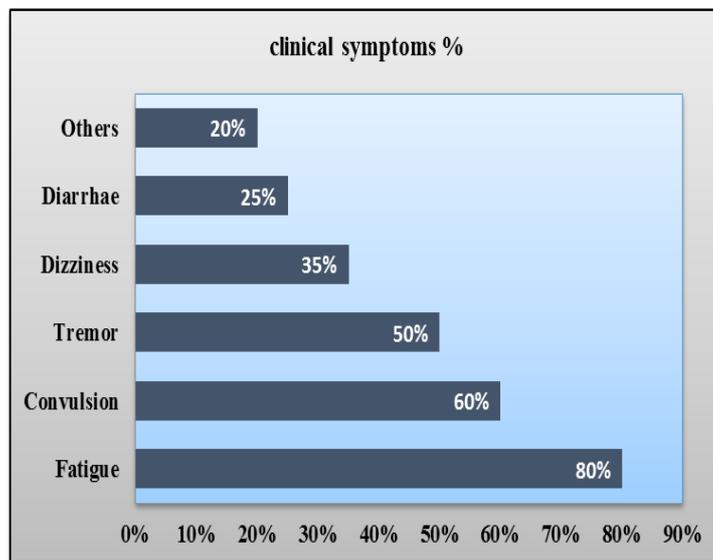


Fig 1: Showing frequency of clinical symptoms in treated animals exposed to IM during experimental studies

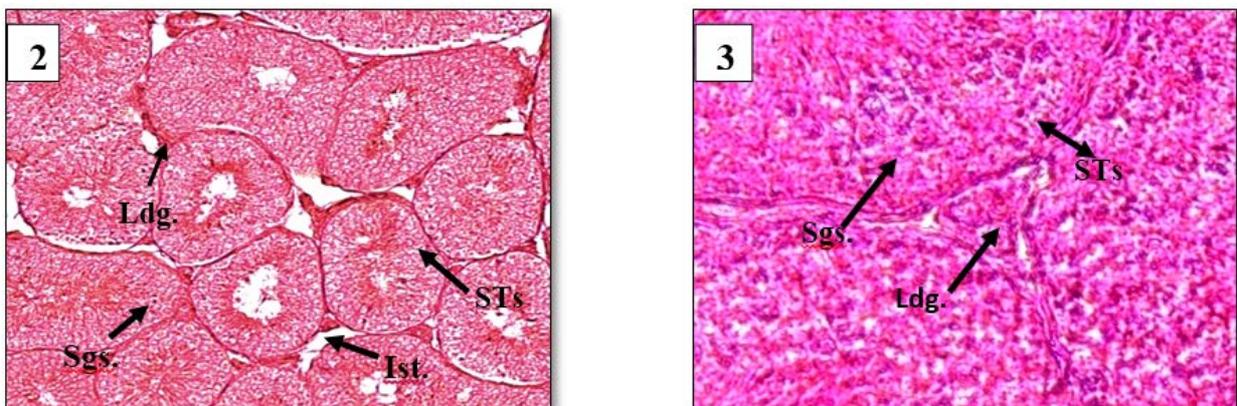


Fig 2-3: Histopathological sections of testis of control group rabbits showing (2), oval shaped seminiferous tubules containing different types of spermatogenic cells with normal Interstitial space (Ist.), interstitial space containing numerous leydig's cells (Ldg.), (3), Majority of cells present in the seminiferous tubules (STs.) are spermatogenic cells (Sgs.) and leydig's cells are clearly visible.(X 10, 63).

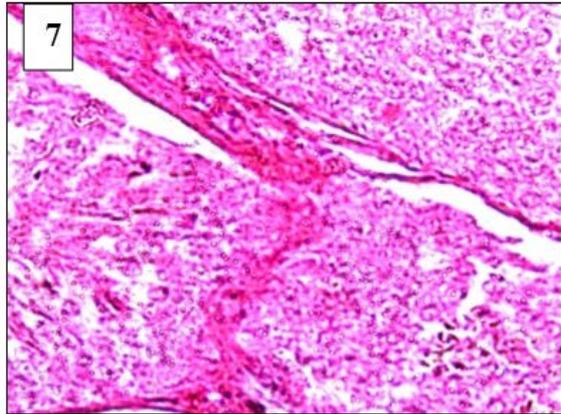
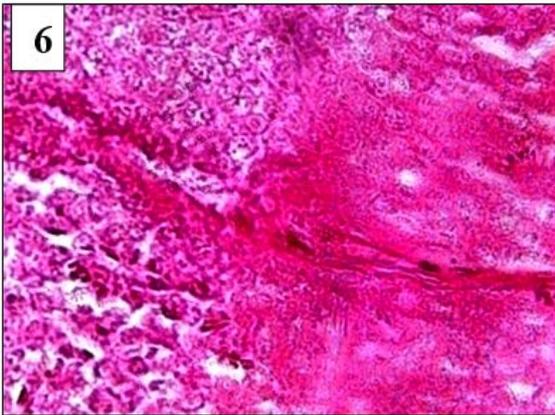
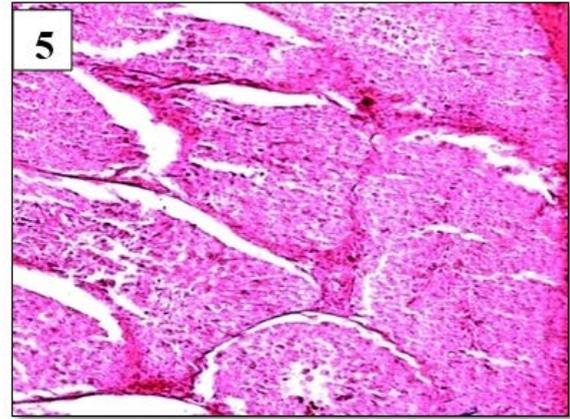
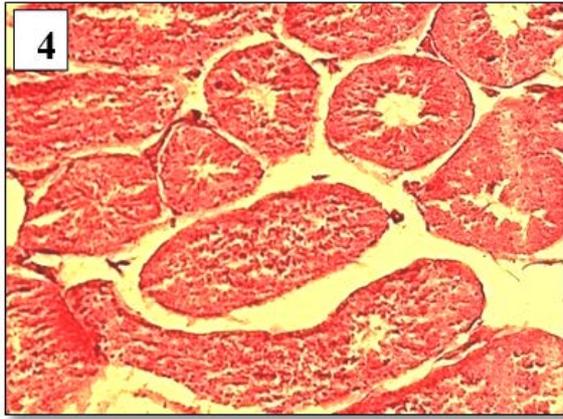


Fig 4-7: Histopathological sections of testis of treated group rabbits showing (4), widened Ist., in few tubules this space has become compressed having a small number of Ldg., (5), vacuolation is present inside STs (6-7), less number of Sgs. are present in the STs with tumor/hypertrophy formation (X 10, 40, 63).

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